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Inter-relationships between composition, physicochemical properties and functionality of lecithin ingredients

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ABSTRACT

Background: Lecithin is widely used as an ingredient in the food industry due to its diverse functionality, mainly attributed to phospholipids (PL), the principal constituents. However, a systematic understanding of the functional properties of lecithin ingredients is missing in the literature.

Scope and approach: This review outlines recent developments in lecithin from botanical origin and reviews the complex inter-relationships between physicochemical properties of PL in lecithin and selected techno-functional properties in micelles, liposomes and oil-in-water emulsions.

Key findings and conclusions: Attributed to their polar phosphatide group and non-polar fatty acids, PL have specific molecular geometries, dissociation constants and charge, which strongly influence their functional properties in micelles, liposomes and oil-in-water emulsions. The PL profile and extrinsic factors (e.g., water, oil, hexane) influence the formation of micelles during separation of lecithin from oil using membrane filtration. In liposomes, PL profile and the presence of surface modifiers (i.e., sterols) affect the particle size and encapsulation efficiency. In emulsion systems, PL and their interaction with minerals and other functional ingredients (e.g., proteins), influence the particle size and physical stability of the oil droplets. This work provides an integrated review of the links between the composition and physicochemical properties of PL, and in turn, scientifically underpins the links between physicochemical and functional properties of lecithin.

1. Introduction

Over recent decades (1990–2020) the global lecithin market has been increasing and is expected to reach 1.59 billion US dollars by 2023, corresponding to an annual growth rate of 7% between 2018 and 2023 (Markets & Markets, 2019). The increasing interest in lecithin as a value-added ingredient is attributable to its unique techno-functional properties, making it suitable as an additive, emulsifier and lubricant in a broad range of applications in pharmaceutical, cosmetics, paint and the food industry (Bueschelberger, 2004; van Nieuwenhuyzen & Tomás, 2008). According to most definitions, lecithin is a mixture of phosphatides obtained by physical procedures from animal or vegetable foodstuffs and is regulated as a food ingredient by the Directive 95/2/EC and 96/77/EC under the number E322 (95/2/EC; 96/77/EC). The legal and technical purity requirements include acetone insoluble (AI), which represents the amount of phospholipids (PL), glycolipids and carbohydrates, and hexane insoluble (HI), which is a measure of impurities, such as fibre. According to the international legislative standards, AI and HI levels have to be greater than 60% and lower than 0.3%, respectively. Additional key quality parameters include loss on drying (<2%), acid value (35 mg/KOH for lecithin and 45 mg/KOH for hydrolyzed lecithin), peroxide value (<10 meq/kg), arsenic (<3 mg/kg), lead (<2 mg/kg) and mercury (<1 mg/kg) (95/2/EC; 96/77/EC; van Nieuwenhuyzen & Tomás, 2008). Lecithin can be obtained from botanical (e.g., soybeans, sunflower seeds and rapeseed) or animal (e.g., egg yolk, cheese whey, fish) sources, with this review focusing on lecithin from botanical sources only. Among the botanical sources, soybeans are by far the most important commercial source of lecithin, accounting for more than 80% of worldwide production (van Nieuwenhuyzen & Szuhaj, 1998). Sunflower and rapeseed also represent good alternatives to soy lecithin, owing to their similar composition and the advantage of being free of any genetically modified source material (van Nieuwenhuyzen & Szuhaj, 1998). As far as sunflower lecithin, the fatty acid composition, and the pleasant nutty flavour, support its use in Europe as an alternative source to soy lecithin (van Nieuwenhuyzen and Tomás, 2008). The global market for sunflower and rapeseed lecithin is expanding, with

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highest growth levels for sunflower lecithin in Argentina, France, Hungary, Ukraine and Russia, while the Indian market for rapeseed lecithin is also growing strongly (Guiotto, Tomás, & Diehl, 2015; List, 2015).

In addition to the aforementioned botanical sources, recent studies have demonstrated the opportunity to extract lecithin from camelina seed (*Camelina sativa* L. crantz) (Belayneh, Wehling, Cahoon, & Ciftci, 2018), corn (Liu et al., 2018) and rice bran (Jala & Prasad, 2015; Sun, Zhang, Tian, Yang, & Xie, 2020). Despite the availability of other botanical sources, the industrial applications of rice bran, for example, are limited, due mainly to the low extraction efficiency (Jala & Prasad, 2015).

This review aims to provide a comprehensive, integrated analysis of lecithin, with an emphasis on PL, as the principal chemical constituents, linking botanical origin, manufacturing approaches and physicochemical properties, through to selected applications (i.e., micelles, liposomes, emulsions), in a total chain, food systems, approach. The composition-structure-functional relationships, along with mechanistic insights into the interfacial properties of lecithin and PL, will be reviewed. This novel work will support formulation scientists in identifying and controlling the factors influencing the functionality of lecithin as an ingredient in food applications.

2. Lecithin

2.1. Manufacture

Lecithin is a co-product of the vegetable oil industry and is extracted from crude oil through sequential unit operations, which include degumming, drying and cooling (Bueschelberger, 2004; van Nieuwenhuyzen & Tomás, 2008) (Fig. 1). Degumming represents the key unit operation for ensuring efficient extraction of lecithin from crude oil and achieving high purity (i.e., high AI values), together with high physicochemical stability of the oil. Water and acid degumming are the two most established conventional approaches used to degum vegetable oils, and over the last decade, enzymatic degumming and membrane filtration have also been applied (Sharma, Yadav, & Upadhyay, 2019). In water degumming, water (1–3%) at 50–70 °C is added to the crude oil and the mixture is stirred for a maximum of 1 h. In acid degumming, different acid solutions (i.e., acetic, tartaric, phosphoric or citric acid) can be added to the crude oil, with citric acid most commonly used. During the degumming step, 2% of diluted citric acid (i.e., 30%) is mixed in crude oil for up to 30 min, followed by the addition of water (i.e., 0.5–3% water at 40 °C) (Sharma et al., 2019). Enzymatic degumming is of interest due to the improved yield compared to the more conventional degumming methods (Sharma et al., 2019). The most commonly used enzymes are phospholipase A_1 (PLA_1) and phospholipase C (PLC), with PLA_1 hydrolysing PL into lysoPL and free fatty acids, while PLC hydrolyses PL into diacylglyceride and phosphate ester (Bueschelberger, 2004; Joshi, Paratkar, & Thorat, 2006; van Nieuwenhuyzen & Tomás, 2008). While very effective, more widespread application of enzymatic degumming is limited due to the high cost of the process (Sharma et al., 2019). Recently, membrane filtration technology, using ultrafiltration membranes, with 20 kDa molecular weight cut off, has been investigated for degumming of lecithin. Such filtration-based approaches to degumming of lecithin have the advantage of reduced volumes of effluent and oil losses occurring by trapping with gums, being more effective than the aforementioned processes in total gum removal (Sharma et al., 2019). However, the adoption of membrane filtration at industrial scale is extremely limited, due mainly to the long processing time and the decrease in membrane performance caused by fouling over time. After the degumming step during lecithin manufacture, lecithin is dried to a moisture content less than 1% and is typically microbiologically stabilized by adding hydrogen peroxide.

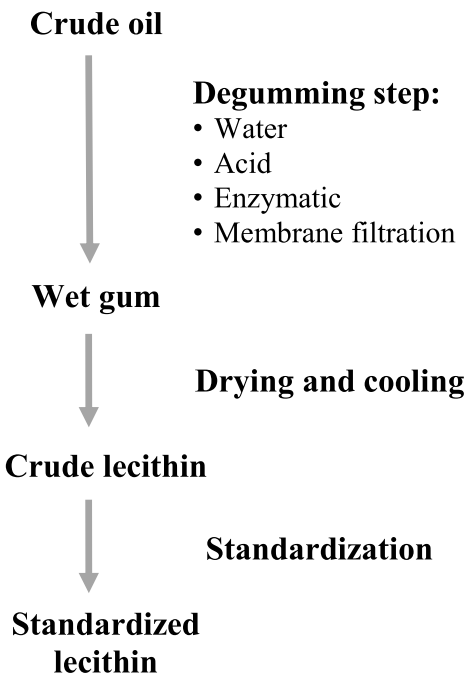


Fig. 1. Schematic representation of the steps involved in preparation of lecithin from crude oil.

In addition to the treatments reported above, chemical modifications, fractionation and compounding can be used to modify the chemical composition of lecithin ingredients, and thus tailor their functional properties, which will be reviewed in the following sections. Chemical modifications include hydroxylation, acetylation and hydrogenation and are currently used in non-food applications (e.g., cosmetic industry) (Bueschelberg, 2004; Joshi et al., 2006). Fractionation allows the isolation of selected fractions of lecithin and encompasses (i) de-oiling with acetone to remove the triglycerides and increase the PL content; (ii) fractionation with alcohol to extract selected PL, such as PC, which has the highest solubility of all PL in alcohol. Compounding of lecithin consists of the addition of oils and it is mainly used to adjust the viscosity values in a range between 5 and 10 Pa s (at 25 °C) to achieve better control of down-stream processing steps (Bueschelberg, 2004).

2.2. Composition

Lecithin mainly contains phospholipids (PL; ~50%) and each botanical source is characterized by a specific PL profile, which is influenced by botanical source variety, geographical region and therefore weather and storage conditions, in addition to exact manufacturing procedure (e.g., use of water versus acid degumming) (Bueschelberg, 2004). In soybean lecithin, the PL composition includes 15% phosphatidylcholine (PC), 10% phosphatidylinositol (PI), 11% phosphatidylethanolamine (PE), and 4% phosphatidic acid (PA) (van Nieuwenhuyzen & Szuhaj, 1998). A similar PL profile can be found in sunflower and rapeseed lecithin, with sunflower lecithin containing 16% PC, 8% PE, 14% PI and 3% PA, while rapeseed contains 17% PC, 9% PE, 10% PI, 6% PA (Nguyen et al., 2014; van Nieuwenhuyzen & Szuhaj, 1998) (Table 1). Additional botanical sources of lecithin are represented by camelina seeds (*Camelina sativa* L. crantz), rice bran and corn. In lecithin from camelina seeds, only traces of PC + lysoPC can be found, while the most abundant PL is PI (25–38%), followed by lysoPL (5–12%) and PE + lysoPL (4–6%) (Belayneh et al., 2018). In contrast, rice bran lecithin contains 33–35% PC, 17–20% PI, 12% PE and 20–23% PA (Sun et al., 2020).

In lecithin ingredients, triglycerides account for approximately 34% of total mass and different fatty acid profiles can be observed between soy, sunflower and rapeseed lecithin (Table 1). In soy lecithin, linoleic

Table 1
Phospholipids and fatty acids in soy, sunflower and rapeseed lecithin. Modified from van Nieuwenhuyzen and Tomás (2008).

Composition	Lecithin source		
Phospholipids (%)	**Soy**	**Sunflower**	**Rapeseed**
PC	15	16	17
PE	11	8	9
PI	10	14	10
PA	4	3	4
Fatty acids (%)			
16:0	16	11	7
18:0	4	4	1
18:1	17	18	56
18:2	55	63	25
18:3	7	0	6

(C18:2), oleic (C18:1), palmitic (C16:0) and α-linolenic (C18:3) acids account for 55, 17, 16, and 7%, respectively, of the total fatty acids (van Nieuwenhuyzen & Tomás, 2008). A similar fatty acid profile can be observed in sunflower lecithin, while in rapeseed lecithin higher and lower proportions of oleic (56%) and linoleic (25%) acids are typically found (Table 1).

In addition to the principal compounds present in lecithin, smaller amounts of more minor substances, including glycolipids (e.g., sterol glycoside, acyl steryl glucoside, digalactosyldiacylglycerol, monogalactosyldiacylglycerol), sterols, tocopherols, free fatty acids, oligosaccharides, proteins, carotenoids and Maillard reaction products and minerals (e.g., calcium and magnesium) (Nguyen et al., 2014; van Nieuwenhuyzen & Tomás, 2008) are also present. As an example of the proportion and diversity of minor compounds, soybean lecithin powder has been reported to contain approximately 10% glycolipids (primarily acyl-sterylglycosides and sterylglucosides) and 7.5% oligosaccharides, stachyose, sucrose and raffinose (Kang, Yin, & Cao, 2019; Scholfield, Dutton, & Dimler, 1952). Residual plant proteins with molecular weight ranging from 10.5 to 52.2 kDa have also been detected in commercial lecithin ingredients at concentrations of 100–1400 mg/kg (Martín-Hernández, Bénet, & Marvin-Guy, 2005).

3. Physicochemical properties of phospholipids

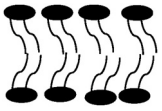
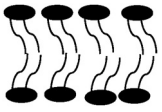
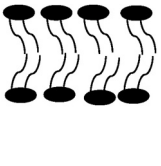
3.1. Chemical structure of phospholipids: structure and molecular charge

PL, the main constituents of lecithin ingredients, consist of a glycerol backbone esterified at positions sn-1 and sn-2 with fatty acids, and in position sn-3 with a phosphate. The fatty acids confer to the PL a lipophilic character and generally, saturated fatty acids are preferentially esterified at the sn-1 position, while unsaturated fatty acids tend to be esterified at the sn-2 position (Cui & Decker, 2016). In position sn-3, a phosphate group esterified with a specific functional group (Table 2) confers the hydrophilic characteristic to the PL. In addition, according to the functional group esterified to the sn-3 position, the following PL arise: PC, PI, PE, PA. The chemical structure of PL, and their physicochemical properties, strongly influence the partition coefficient in oil and water and therefore the amount of individual PL extracted during the degumming step and, as a consequence, the physicochemical stability of the crude oil. These characteristics of individual PL are described in detail in the following sections.

3.1.1. Phosphatidylcholine

Phosphatidylcholine (PC) contains a choline moiety that has a negative charge on the phosphate group and a positive charge on the trimethyl-amino group (Table 2). At pH < 3.5, which is the pH at which the molecule is exactly half dissociated (i.e., pK_a), the phosphate group will lose the negative charge, resulting in a more hydrophilic PC molecule and during degumming the partitioning coefficient will strongly be on the water side in the oil-water system (Dijkstra, 2017; Tatulian, 2012). Moreover, even at pH > 5, when the phosphate group carries a negative charge, PC remains hydrophilic because, for steric reasons, it forms an internal salt with the quaternary amino group. Therefore, PC is

Table 2
Physicochemical properties of phospholipids. Modified from Dijkstra (2017) and Li et al. (2015).

Phospholipid	Functional group	Charge of PL at different pH					Molecular geometry	Structure at the interface	
		2	4	5-7	8-9	10			
Phosphatidylcholine (PC)	CH_2CH_2-$N^+(CH_3)_3$	+	(±)	±	±	±	Cylinder		Lamellar
Phosphatidylinositol (PI)	$C_6H_{11}0_5$	(0)	(-)	-	-	-	Cylinder		Lamellar
Phosphatidylethanolamine (PE)	CH_2CH-NH_3^+ COO^-	+	(±)	±	±	-	Cone		Hexagonal
Phosphatidic acid (PA)	H	0	(-)	-	(2-)	2-	Cylinder		Lamellar

Charges between parentheses (e.g., (−)) indicate a transition between the value at lower pH and the value at higher pH.
When the pH is increased, more phosphate groups dissociate and a zwitterion (indicated by ±), in which the positive amino group forms an internal salt with the negative phosphate group, is formed.

hydrophilic at all the pH values, thereby enabling a high extraction yield (Dijkstra, 2017).

3.1.2. Phosphatidylinositol

In a similar manner, phosphatidylinositol (PI) possesses five hydroxyl groups, which make the molecule strongly hydrophilic (Table 2). At pH less than its pK_a value of 3.5, the negative charge is lost, conferring a hydrophilic character on the molecule (Dijkstra, 2017), while at neutral pH the phosphate group is dissociated. During water degumming, PI in crude oil will be hydrated and therefore its content in oil efficiently degummed using water is negligible. For both PC and PI, the chemical properties described above are exploited during degumming (Tatulian, 2012) and it has been shown that the degumming conditions strongly influence the proportions of individual PL in lecithin. Li, Pedersen, Anankanbil, and Guo (2018) reported that, in soy lecithin obtained by water degumming, the proportions of PC and PI were higher than in chemically degummed lecithin.

3.1.3. Phosphatidylethanolamine

Phosphatidylethanolamine (PE) contains an amino group and a phosphate group (Table 2). At neutral pH, the amine group is protonated and the phosphate group is dissociated. Molecular structural analysis shows that the negative phosphate oxygen can be quite close to the positive amino nitrogen in the six-membered ring. Therefore, the dipole moment of the molecule becomes quite small, and, as a consequence, PE is poorly hydratable. During acid degumming, the phosphate group in the PE molecule is protonated and the molecule has a positive charge, making it hydrophilic. Similarly, when pH is increased (as during alkali refining), the PE molecule loses its positive charge, making the molecule more hydrophilic (Dijkstra, 2017).

3.1.4. Phosphatidic acid

Phosphatidic acid (PA) represents the simplest PL, with a hydrogen atom as functional group (Table 2) and two hydroxyl groups that can dissociate. The pK_a value of the non-dissociated and dissociated acid is about 2.7–3.8 and 7.9–8.7, respectively. Under acidic pH conditions, the hydroxyl groups of its phosphate moiety will not dissociate, since the pK_a is in the range 2.7–3.8 and therefore the molecule possesses a strong hydrophobic character. When the pH is raised to alkaline conditions the hydroxyl groups present in PA are dissociated and PA possess a negative charge, associated with high hydrophilicity. PA is also present as calcium or magnesium salts and it is strongly lipophilic (Dijkstra, 2017).

3.1.5. Lysophospholipids

Beside the above-mentioned PL, lysophospholipids (lysoPL) are also present in lecithin. LysoPL consist of phospholipids from which a fatty acid chain has been removed from either the sn-1 or sn-2 position and are predominantly the products of enzymatic reactions (PLA1 and PLA2) from the hydrolysis of the ester bond on the glycerol moiety in the phosphatide (Garti, 2002). By sharing similar chemical properties to the corresponding PL, the presence of only a fatty acid and a free hydroxyl group confers to the molecule a stronger hydrophilic character than the corresponding PL. As reported in the above sections, the chemical structure and properties of the single PL are important in choosing the most appropriate approach for degumming of lecithin. During the degumming step, changes in pH are largely exploited with the aim of changing the partitioning coefficient between oil and water of PL, thereby increasing the extraction of lecithin as well as the physicochemical stability of crude oil (Dijkstra, 2017). While the link between the physicochemical properties of PL and the respective partitioning coefficients are described in the literature in qualitative terms (Dijkstra, 2017), to the best of our knowledge, no measurements have been reported in the literature. Further research in this area should investigate these linkages more deeply and determine the effects of different degumming conditions on the chemical composition of the resultant lecithin ingredients.

3.2. Molecular geometry, packing parameter and hydrophilic-lipophilic balance

The chemical structure of PL strongly influence their molecular geometry (Table 2), which in turn, will influence their techno-functional properties, such as self-assembly and emulsification. According to the physicochemical properties of the PL, different molecular geometries of the PL in aqueous environment can be identified; most of the PL, including PC, PI and PA form cylindrical shapes, while PE and lysoPL form cone and inverted cone shapes, respectively (Table 2). At the interface of multiphase systems, PC forms a lamellar structure, with well-ordered mono- and bi-layers and is therefore of particular importance for stabilization of oil-in-water emulsions. Phosphatidylethanolamine (PE) forms reversed hexagonal structures, which are more challenging to arrange at interfaces (Table 2). In contrast, the hydrolyzed lysophosphatidylcholine (lysoPC), and likely also lysophosphatidylethanolamine (LysoPE), form hexagonal wide spread clusters (Bueschelberger, 2004; Li et al., 2015; van Nieuwenhuyzen & Szuhaj, 1998).

The molecular geometry of a surfactant molecule can be characterized by a packing parameter (p), which is equal to the ratio of the tail group (aT) to head group cross-sectional areas (aH):

$$p = \frac{aT}{aH} \tag{1}$$

The packing parameter determines the optimum packing of surfactants when they assemble into monolayers, which in turn determines the optimum curvature that tends to be adopted by a given surfactant (Israelachvili, 2011). When the head group is appreciably larger than the tail group ($p < 1$), then the monolayer adopts a curvature where the head groups point outward, which favours the formation of oil-in-water emulsions and micelles. Conversely, when the tail group is appreciably larger than the head group ($p > 1$), then the monolayer adopts a curvature where the tail groups point outwards, which favours the formation of water-in-oil emulsions and reverse micelles. In addition, if the head group and tail group cross-sectional areas are similar ($p = 1$), then the monolayer tends to be planar, which favours the formation of bilayers and vesicles.

The hydrophilic-lipophilic balance (HLB) is determined by the head group and the fatty acids present. It is generally accepted that hydrophilic surfactants have high HLB values (>10), whereas lipophilic surfactants have low HLB values (1–10) (Macedo et al., 2006). Lecithin has HLB numbers ranging between 4, for standardised lecithin, and 7 for lecithin enriched in the PC fraction, which means they can be dispersed in both oil and water phases. In contrast, lecithin with a high proportion of lysoPL tends to have HLB numbers higher than 7 and are therefore usually dispersed in the water phase prior to homogenization. Although the HLB system is valuable and convenient from a product development point of view, its main shortcoming is related to limited information about how a surfactant will perform under different environmental conditions or in systems with different and complex compositions like food, where multiple interactions often occur simultaneously (Garti, 2002; Macedo et al., 2006; McClements, 2015).

4. Functional properties of lecithin

The functional properties of lecithin are strongly influenced by the physicochemical properties and the geometrical structure of PL. In particular, the ability of PL to self-assemble, with reduction of surface tension, make possible the formation of different supra-molecular structures and oil-in-water emulsions, respectively. In the following sections, selected functional properties, including self-assembly and interfacial properties of lecithin and PL will be reviewed in micelles, liposomes and oil-in-water emulsions.

4.1. Micelles

PL present in lecithin can self-associate in water or oil in order to minimize the free energy of the system. The main driving force for the formation of these types of colloidal systems is hydrophobicity, which is the tendency for the system to reduce thermodynamically unfavourable contact between non-polar groups and water (Israelachvili, 2011; Li & Yang, 2015). PL aggregate and form micelle structures above a critical concentration, expressed as the critical micelle concentration (CMC). This parameter is usually determined by measuring the absorbance in the visible range (between 400 and 600 nm) as a function of PL concentration upon the addition of a dye (e.g., merocyanin) or an electron-acceptor molecule (i.e., 7,7,8,8-tetracyano-quinodimethane, TCNQ) and it is determined from the intersection between the regression straight line of the linearly dependent and the plateau regions.

From a structural point of view, PL in water expose their hydrophilic groups, while the hydrophobic fatty acids form the core (direct micelle), whereas in an oil system, the fatty acids are exposed and the hydrophilic group forms the core of the micelles (reverse micelles) (McClements, 2020). Micelle structures have been generally utilized to encapsulate and deliver a wide range of hydrophobic and amphiphilic bioactive compounds (McClements, 2015, 2020). In the pharmaceutical sector, micelle structures containing lecithin ingredients have been used to deliver bioactive compounds through the skin (Kumar & Katare, 2005; Raut et al., 2012; Shaikh, Jadhav, & Kadam, 2015), while in the food sector, to the authors knowledge, these systems have not been exploited. On the other hand, increasing attention is being given to the use of reverse micelles for controlling the extraction efficiency during degumming and oxidative stability of oil (Cui & Decker, 2016; Santori, Di Nicola, Moglie, & Polonara, 2012).

During lecithin degumming, PL form reverse micelles in non-aqueous systems, such as vegetable oils and hexane-oil mixtures, and this has been exploited during degumming by membrane filtration (Sharma et al., 2019). Nonporous polymeric hydrophobic membranes with an active layer (polymethylsiloxane) showed high performance in degumming processing across a wide range of PL content and systems (Manjula, Kobayashi, & Subramanian, 2011); however, ultrafiltration (UF) membranes are generally preferred for their higher productivity and rejection performance (Saravanan, Bhosle, & Subramanian, 2006; Subramanian, Nakajima, Raghavarao, & Kimura, 2004). The efficiency of membrane separation has been attributed to the formation of reverse micelles, which are separated by size exclusion and chemical interactions between membrane surfaces and chemical compounds (i.e., PL, triglycerides) present in the oil. In oil and oil-hexane systems, the CMC of PL is dependent on several interlinked factors, which include the system (i.e., oil or oil-hexane mixtures), moisture content, oil composition and PL profile.

The composition of the system (oil or oil-hexane mixture) plays a fundamental role in micelle formation (Table 3). Lower CMC values have been observed in systems containing hexane and oil, as opposed to oil alone, due to the higher hydrophobicity of hexane than oil. It has also been shown that in oil-hexane systems, the CMC of PC is much lower (70 mg/kg) than in oil alone (440 mg/kg) (Manjula et al., 2011; Subramanian et al., 2004). Similarly, in mixed PL systems, the CMC in hexane system was 520 mg/kg, which was much lower than the CMC of mixed PL in oil alone (850 mg/kg) (Manjula et al., 2011). The presence of moisture in oil-hexane systems containing PL also influences micelle formation and it has been observed that CMC decreased from 520 to 430 mg/kg with an increase in the moisture content from 100 to 800 mg/kg in the system (Manjula et al., 2011). In a lecithin-oil-hexane system, a large decrease in CMC of mixed PL from 330 to 110 mg/kg was measured when the addition of water was increased from 100 to 1800 mg/kg (Hancer, Patist, Kean, & Muralidhara, 2002). The actual PL profile in crude oil or hexane-oil systems represents another important factor influencing the formation of mixed micelles; for example, the CMC of PC in oil was lower (440 mg/kg) than that measured in mixed PL systems (850 mg/kg) (Manjula et al., 2011). Similarly, in refined soy oil, the CMC of soy lecithin (PC content 23.6%) was lower (850 mg/kg) than in crude soy oil (1020 mg/kg) (Subramanian et al., 2004). A similar effect of PL composition can be observed in a system containing hexane-soybean oil (70:30) and PA in the sodium salt (hydratable) or calcium salt (nonhydratable) forms, with the results showing that the CMC of the hydratable form was lower (70 mg/kg) than the non-hydratable PL (180 mg/kg) (Hancer et al., 2002). The oil content in lecithin also affects the hydrophobicity of the system, and thus, micelleformation. It has been observed that when de-oiled lecithin is

Table 3
Factors influencing the critical micelle concentration (CMC) of lecithin during degumming process and oxidative stability of oil.

Application field	System	Main findings	References
Degumming step during vegetable oil extraction	- Oil - Hexane-oil mixture	CMC values are influenced by - System composition: CMC lower in hexane-oil than oil system CMC of PC in hexane (70 mg/kg) *vs* oil (440 mg/kg) CMC of PL in hexane (520 mg/kg) *vs* oil system (850 mg/kg) - Amount of water in system containing hexane: CMC decreased with increasing amount of water CMC values: 520 mg/kg with 100 mg/kg water 430 mg/kg with 800 mg/kg water - PL composition: CMC lower with high amount of PC or hydratable PL CMC values of soy lecithin in refined soy oil: 850 mg/kg *vs* crude soy oil system 1020 mg/kg In hexane-soy bean oil (70:30) with hydratable PA (PA, sodium salt) (70 mg/kg) *vs* nonhydratable phospholipid (PA, calcium salt) (180 mg/kg) - Amount of oil: CMC higher with deoiled lecithin in hexane-oil systems CMC was 520 mg/kg in non-de-oiled lecithin and 460 mg/kg de-oiled lecithin	Hancer et al., 2002; Manjula et al., 2011; Subramanian et al., 2004
Oil stability	Oil	Oil stability is negatively influenced by the presence of PL/lecithin CMC values are influenced by - type of oil: MCT: 3890 ppm olive oil: 1923 ppm soybean oil: 1593 ppm corn oil: 1288 ppm rapeseed oil: 1973 ppm - moisture: low CMC at low moisture level in MCT at 100 ppm of moisture, CMC was 2610 ppm in MCT at 900 ppm of water, CMC was 4407 ppm - oxidized products: CMC decreased with increasing of oxidized products CMC 800 ppm after 4 h heating at 180 °C CMC 200 ppm after 120 h heating at 180 °C	Kim et al., 2015; Kim et al., 2018; Kim et al., 2019

dissolved in hexane and used in the determination of CMC of mixed PL, the CMC value was slightly lower (460 mg/kg) than that of the system containing lecithin and oil (520 mg/kg) (Manjula et al., 2011).

The formation of PL micelles not only influences the filtration performance and extraction efficiency, but also strongly affects the oxidative stability, measured by a wider range of indicators such as head space oxygen content, conjugated dienoic acid, and *p*-anisidine value or other secondary oxidation products in the extracted oil. In crude oil, lipid oxidation mainly takes place at the oil-water micelle interface, which is constituted by PL, together with other amphiphilic compounds, (e.g., oxidation products, free fatty acids, phospholipids, diacylglycerols, monoacylglycerols and antioxidants). Type of oil, moisture content and the presence of oxidized products influence the CMC in bulk oil systems. In medium-chain triglyceride (MCT) oil, lecithin had a CMC value of 3890 ppm, which is significantly higher than those reported for other vegetable oils, including olive (1923 ppm), soybean (1593 ppm), corn (1288 ppm) and rapeseed (1973 ppm) oils. The high CMC in MCT may be attributed to the absence of amphiphilic compounds, which may help to form micelle structures at high PL content. In addition, the presence of moisture strongly influenced the CMC; in MCT and corn oil, the CMC value for lecithin was 2610 ppm at 100 ppm of moisture, increasing to ~4407 ppm when the moisture content in MCT increased to 900 ppm. In bulk oil, lipid oxidation products, measured as secondary oxidation products (i.e., total polar material) strongly decrease the CMC of lecithin and it has been observed that after heating for 4 h at 180 °C, CMC of lecithin in corn oils decreased to 58.2%, and after 120 h, the CMC had decreased to 13.9% of the original value. This indicates that the presence of oxidized products after lipid oxidation strongly decreases the CMC of lecithin (Kim, Kim, & Lee, 2018). The presence of lecithin influenced also the oxidative stability of corn oil upon the physical separation process where components are removed from a liquid by a vapour stream (i.e., stripping treatment) (Kim, Kim, Yi, Oh, & Lee, 2015). Lecithin-supplemented non-stripped corn oil, near and above the CMC of 1288 ppm, had high oxidative stability. Non-stripped corn oil supplemented with lecithin at concentration close to the CMC value showed the highest oxidative stability, which could be due to the incorporation of tocopherols into the micelles formed by the added lecithin. In stripped corn oil, CMC of lecithin is 1790 ppm; however, higher antioxidant properties determined using the DPPH assay have been observed at low lecithin addition (600 ppm) than at higher concentrations (1200–3000 ppm) (Kim, Woo, Ryu, Kim, & Lee, 2019).

4.2. Liposomes

Liposomes are colloidal particles where the hydrophilic head groups of the PL molecules are orientated towards the aqueous phase, while the hydrophobic region of each of the monolayers face each other in the middle of the bilayer vesicle to avoid contact with water molecules (Khorasani, Danaei, & Mozafari, 2018). When PL are exposed to an aqueous environment they minimize the contact of their hydrophobic groups (fatty acids) with the water molecules and arrange themselves in the form of bilayer vesicles *via* Van-der Waals and hydrophobic/hydrophilic interactions. According to the ability of PL to arrange themseves, unilamellar vesicles with a single PL bilayer surrounding the aqueous phase and particle size >20 nm (determined using a dynamic light scattering technique), or multilamellar liposomes with several unilamellar vesicles separated by the layers of water can be formed (Akbarzadeh et al., 2013; Shishir, Karim, Gowd, Zheng, & Chen, 2019). Within the lipophilic (fatty acids) or hydrophilic (polar head) phase of the liposome, bioactive compounds can be incorporated with high encapsulation efficiency, defined as the ratio between the amount of bioactive compound present upon centrifugation (i.e., encapsulated) and initial amount added. Several studies have demonstrated that in liposomes formulated with lecithin, the encapsulation efficiency of bioactive compounds such as β-carotene, nisin, lactoferrin, curcumin, tea polyphenols is higher than 60% (Gülseren & Corredig, 2013; Imran et al., 2015; Michelon, Mantovani, Sinigaglia-Coimbra, de la Torre, & Cunha, 2016; Peng, Zou, Liu, Liu, & McClements, 2018; Vergara & Shene, 2019).

Liposomes encapsulating bioactive compounds have initially been developed for use in the pharmaceutical field (Le et al., 2019). Soy lecithin liposomes containing ethanol as penetration enhancer have been shown to be effective in the transdermal delivery of herbal drugs with antimicrobial properties (Biswas, Chattopadhyay, Banerjee, & Bandyopadhyay, 2002; Singh, Vengurlekar, & Rathod, 2014) or drugs used for the topical treatment of alopecia (Mura, Pirot, Manconi, Falson, & Fadda, 2007).

The use of liposome-based technology has been further developed in the food area and a number of studies have shown the opportunity to incorporate liposome containing bioactive compounds in orange juice (vitamin C and E), cheese (polyphenols) and active packaging (quercitin and rutin) to improve flavour release and oxidative stability, as well as for extending the shelf-life of food products (Marsanasco, Márquez, Wagner, del Valle Alonso, & Chiaramoni, 2011; Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2014, 2016; Silva-Weiss et al., 2018). However, their use in food applications is generally limited by cost, use of organic solvent, scale-up and low encapsulation efficiency compared to other structures (i.e., oil-in-water emulsions) (Liu, Ye, & Singh, 2015).

Several technological approaches can be applied for the preparation of liposomes containing PL. For instance, thin layer thin-film hydration method or ethanol/ether injection, which include the use of organic solvent (Bryła, Lewandowicz, & Juzwa, 2015; Michelon et al., 2016; Silva-Weiss et al., 2018; Tai et al., 2018; Vergara & Shene, 2019) or high-shear mixing and high-pressure homogenization (Gülseren & Corredig, 2013; Peng et al., 2018; Wang, Acevedo, & Marangoni, 2017) have been reported as efficient strategies for liposome formation. However, approaches involving the application of mechanical energy are highly preferred in the food sector over the use of organic solvents due to the absence of solvents, the relatively simple downstream processing steps required and the potential for scale up (Jahn, Vreeland, DeVoe, Locascio, & Gaitan, 2007; van Swaay & deMello, 2013).

The technological and functional properties of liposomes are strongly influenced by the PL profile. Highly stable liposomes can be obtained with synthetic PL (i.e., dipalmitoylphosphatidylcholine, DPPC and dimyristoylphosphatidylcholine, DMPC), which possess PC as PL and saturated fatty acids (i.e., palmitic and myristic acid) (Li et al., 2015). Lecithin from botanical sources offer the advantages of being less expensive than synthetic PL, but the presence of a diverse population of individual PL types and highly unsaturated fatty acids cause modifications of the physicochemical properties of liposomes and make the role of individual compounds in the physicochemical features more complex to understand. In liposomes containing sunflower lecithin with PC content ranging from 50 to 90% of the total PL, the particle size distribution upon microfluidization ranged between 10 and 1000 nm and no significant differences were observed within the samples with increasing amount of PC (Peng et al., 2018). A more significant role of the PL profile in soy lecithin ingredients can be found in liposomes formulated with soy lecithin ingredients upon a de-oiling step with acetone, which facilitates selective fractionation of the PL fraction in obtaining a lecithin ingredient with higher proportions of PC and PI than the untreated equivalent (Taladri et al., 2017). It has been demonstrated by Taladri et al. (2017) that when liposomes are formulated with de-oiled lecithin, with approximately a 1.3 fold increase in PC and PI, the particle size, determined by dynamic light scattering, was lower (87 nm) than the untreated counterpart (132 nm). In lecithin ingredients, where no additional treatments have been performed after degumming, both PL and fatty acids play a fundamental role in the physicochemical properties of liposomes. Larger particle size and higher encapsulation efficiency of lactoferrin have been observed in liposomes formulated with rapeseed compared to soy lecithin, with these differences attributed to different chemical composition of the selected lecithin and, in particular, with rapeseed lecithin containing a lower proportion of

polyunsaturated and long-chain fatty acids (34%), together with a higher proportion of PA (126 mg/g) than soy lecithin (57% of polyunsaturated fatty acids and 16 mg/g PA) (Vergara & Shene, 2019).

The PL profile of lecithin also influences the zeta potential, a measure of the strength of electrical charge of mutual repulsion or attraction among particles. Liposomes prepared with soy lecithin had a zeta potential of -58.3 ± 2.2 mV, due to the presence of PI and PA, which carry a net negative charge at pH 6.0–6.5, while liposomes containing mainly PC, which is zwitterionic, had low zeta potential of -3.6 ± 0.4 mV (Zhao, Temelli, Curtis, & Chen, 2015). Low values of zeta potential (in absolute terms) in colloidal systems are usually correlated with low physical stability, thus indicating that liposomes with high PC content may have poor physical stability. However, in liposome systems, zeta potential has been mainly used to understand and predict the physicochemical properties, as opposed to the physical stability of the colloidal particles in such systems.

In addition to the polar head group present in lecithin ingredients, the physicochemical properties of fatty acid chains of PL influence the physical properties of the liposomes, and it has been shown that PL with shorter fatty acid chain lengths (due to the lower packing parameter) are able to form liposomes with smaller particle sizes (Zhao et al., 2015). In liposomes prepared using supercritical carbon dioxide technology and containing PC with increasing fatty acid chain length from 14 to 18 carbon atoms, the particle size increased from 60 to 150 nm (Zhao et al., 2015). In addition, the presence of unsaturated and saturated or hydrogenated fatty acids in PL play a fundamental role in the stability and encapsulation efficiency of micelles. Unsaturated PL displayed poor physical stability and encapsulation efficiency, due to the presence of double bonds in the fatty acid chain, which disarrange the liposome structure with the formation of interspace within the membrane (Grit & Crommelin, 1993), and an increase in the rate of oxidation. On the other hand, saturated or hydrogenated PL exhibit higher phase transition temperatures, gel-phases with a lower membrane fluidity which facilitate high encapsulation efficiency and loading capacity (Sebaaly, Greige-Gerges, Stainmesse, Fessi, & Charcosset, 2016; Tai, Rappolt, Mao, Gao, & Yuan, 2020).

Another formulation factor exploited in tailoring the physicochemical properties of liposomes is the presence of sterols, surface modifiers for the bilayer, which are usually integrated with conventional liposomes (Tai et al., 2018). When sterols are incorporated into the micelles containing unsaturated PL, the particle size increases and it has been observed that cholesterol and ergosterol induced the formation of relatively larger vesicle size than β-sitosterol. The most compact membrane structure was reported for cholesterol-integrated liposomes, which exhibited the strongest intermolecular interactions in bilayer structures, while the ergosterol-incorporated liposomes were the most fragile and fluid (Tai et al., 2018). Similarly, Zhao et al. (2015) reported that 6-ketocholestanol exhibited the lowest vesicle size and polydispersity index, while cholesterol showed the highest particle size and polydispersity index (Zhao et al., 2015).

The PL and fatty acid profile, together with the addition of surface modifiers, are the main factors influencing the physicochemical properties of liposomes, as reported in this section. Also, minor components present in lecithin, such as minerals and proteins, may have the ability to alter the formation and properties of the liposomes due to their charge or interfacial properties; however, to the authors knowledge, these factors have not been taken into consideration and future studies may focus more on these to help better understanding of their roles in influencing the physicochemical properties of colloidal systems containing lecithin.

4.3. Oil-in-water emulsions

Oil-in-water emulsions consist of oil droplets dispersed in an aqueous phase and stabilized by a surfactant (McClements, 2011). In these colloidal systems, PL reduce the surface tension by orientating at the interface, with the fatty acid tails protruding into the oil droplets and the hydrophilic head orientates toward the water (Liu et al., 2015; Pichot, Watson, & Norton, 2013).

Lecithin is largely used in oil-in-water emulsions to encapsulate and deliver polyunsaturated oils (Komaiko, Sastrosubroto, & McClements, 2016) and/or bioactive compounds (e.g., carotenoids) in food (McClements, 2015). In the pharmaceutical sector, oil-in-water emulsions containing lecithin and co-surfactant (e.g., *n*-butanol) have been explored in order to improve the delivery of bioactive compounds (e.g., curcumin) or therapeutic agents (ketoprofen drugs used for the treatment of tendinopathy or arthritic conditions) into/through the skin (Nikolic et al., 2020; Yousef et al., 2019).

The effectiveness of lecithin in forming oil-in-water emulsions is strongly influenced by the energy applied. For instance, when low-energy methods are used, the mean droplet size is usually greater than 10 μm (Komaiko, Sastrosubroto, & McClements, 2015; Table 4). Conversely, high energy processes, such as high shear mixing, allow the formation of oil droplets in the range 10–100 μm (Guiotto, Cabezas, Diehl, & Tomás, 2013; Pan, Tomás, & Añón, 2004), while high-pressure valve homogenizers, microfluidizers or sonicators enable the formation of oil-in-water emulsions with mean particle size less than 500 nm (Heo, Kim, Pan, & Kim, 2016; Lin, Wang, Li, & Wright, 2014; Washington, 1996). The size of the droplets formed during homogenization typically

Table 4
Physicochemical properties of lecithin in oil-in-water-emulsions.

Lecithin sources	Co-emulsifier	Emulsion properties	References
Lecithin from soy, sunflower, rapeseed	/	- High proportion of PC allows to obtain smaller particle size - Zeta potential is generally lower than −50 mV - At low pH the droplet charge becomes progressively less negative and the particle size increase - The addition of ions (NaCl, $CaCl_2$) causes an increase in particle size	Chung et al., 2017; Chung et al., 2018; Chung et al., 2019; Komaiko et al., 2016: Liang et al., 2017; Ozturk et al., 2014; Washington, 1996
Lysolecithin from soy, rapeseed, sunflower, *Camelina sativa*	/	- Particle size decreases with high proportion of LysoPL - Physical stability improves with high proportion of LysoPL	Belayneh et al., 2018; Guiotto et al., 2013; Komaiko et al., 2015; Pan et al., 2004; Xie & Dunford, 2017
Lecithin from soy	Dairy proteins (casein, whey)	- Particle size decreases when dairy protein are combined with lecithin - Zeta potential is slightly more negative compared with the use of dairy protein alone - High heat stability - High physical stability in presence of minerals or coffee solutions	García-Moreno et al., 2014; Xue & Zhong, 2014; Yesiltas et al. (2019)
	Plant proteins	- Particle size decreases when lecithin and pea protein are blended	Walia and Chen (2020)

decreases with increasing homogenization pressure and lecithin content (Cabezas, Madoery, Diehl, & Tomás, 2012; Guiotto et al., 2013; Komaiko et al., 2016; Mezdour, Desplanques, & Relkin, 2011; Washington, 1996).

The physicochemical properties of oil-in-water emulsions are strongly influenced by lecithin composition and, in particular, the proportion of PL (Table 4) (Ozturk, Argin, Ozilgen, & McClements, 2014; Washington, Chawla, Christy, & Davis, 1989). Lecithin enriched with PC has been shown to be particularly effective at stabilizing lipid droplets (Cabezas et al., 2012; Komaiko et al., 2015) due to the formation of well-ordered lamellar monolayers or bilayers around lipid droplets that can facilitate emulsion formation. PE tends to assemble into reversed hexagonal structures, which are more difficult to form around lipid droplets and more sensitive to pH because of their zwitterionic nature (van Nieuwenhuyzen & Szuhaj, 1998). Previous studies have shown that higher ratios of PC to PE are more conducive to formation of emulsions with high physical stability, determined by recording the changes in the optical properties over the length of the sample as indices of creaming and coalescence (Guiotto et al., 2013) and lecithin ingredients with a high proportion of lysoPL have enhanced ability to form oil-in-water emulsions (Casado, Martín, Torres, & Reglero, 2012; Choi et al., 2011). Several authors have demonstrated that oil-in-water emulsions (10% oil) containing lecithin (1%) from different botanical sources (soy, rapeseed, sunflower, *Camelina sativa*) upon enzymatic degumming, have smaller particle size, and better physical stability (measured by recording the height of the cream layer), than emulsions formed using lecithin degummed using water (Belayneh et al., 2018; Xie & Dunford, 2017).

The PL profile of lecithin ingredients, together with the pH of the aqueous phase, influence the physical stability of emulsions. Under basic and neutral pH conditions, oil-in-water emulsions formulated with lecithin typically have a relatively high negative charge (between −50 and −60 mV), helping to prevent droplet aggregation by generating strong electrostatic repulsion, and thus, poor physical stability (Chung et al., 2019; Chung, Sher, Rousset, Decker, & McClements, 2017). When the pH is reduced, the droplet charge becomes progressively less negative and consequently aggregation phenomena have been observed. Soy lecithin-coated oil droplets were shown to be stable to aggregation in the pH range 3–8 (i.e., with associated high charge), but were unstable at pH 2, due to a low charge density (Ozturk et al., 2014; Washington, 1996). Similarly, the addition of ions at neutral pH in oil-in-water emulsions containing soy lecithin can result in aggregation of the droplets due to reduction of electrostatic repulsion between droplets (Chung, Sher, Rousset, & McClements, 2018; Komaiko et al., 2016; Ozturk et al., 2014; Washington, 1996). Typically, much lower levels of multivalent ions (approximately 3 mM calcium) are required to promote aggregation than monovalent ions (approximately 100 mM sodium) as the former are more effective at increasing the ionic strength and binding to oppositely charged droplets (Washington, 1996). An interesting application in this area is represented by coffee-based beverages, which are usually prepared by adding coffee (acid environment with minerals present) to an oil-in-water emulsion-based system, often containing lecithin. Chung et al. (2019) demonstrated that upon the addition of coffee to an oil-in-water emulsion (10% MCT) containing lecithin (1%), the emulsion had a particle size of 0.83 μm and was physically stable.

Lecithin is often used in combination with other surfactants, including proteins from dairy or plant origin, to reduce the packing parameter and improve the emulsifying performance and flexibility of the interfacial film. Several studies have demonstrated that in oil-in-water emulsions stabilized by a combination of soy lecithin and dairy proteins (e.g., sodium caseinate, whey protein isolate), the particle size decreased and the zeta potential became slightly more negative compared to emulsions containing dairy proteins alone (García-Moreno, Horn, & Jacobsen, 2014; Xue & Zhong, 2014; Yesiltas, García-Moreno, Sørensen, Akoh, & Jacobsen, 2019). In oil-in-water emulsions (10% fish oil) with soy lecithin (0.5%) and sodium caseinate (0.3%), the formation of sodium caseinate–soy phospholipid complexes, produced a favourable structure and thickness of the interfacial layer, thereby improving emulsion physical stability (García-Moreno et al., 2014). In addition, oil-in-water emulsions containing 0.5% de-oiled soy lecithin and 0.5% sodium caseinate displayed good physical stability between pH 5.5 and 7, while at pH less than 5.5 the emulsion was unstable (Koo et al., 2019). Similarly, emulsions formulated with soy lecithin (1–1.5%) and sodium caseinate (0.5–1.5%) displayed good coffee stability with no feathering or evidence of oiling off (Chung et al., 2019). The combination of lecithin (soy or sunflower) and dairy protein allowed the formulation of infant nutritional emulsion systems with good physical stability to heat treatment (i.e., no significant changes in particle size distribution upon heat treatment) (Drapala, Auty, Mulvihill, & O'Mahony, 2016; Drapala, Auty, Mulvihill, & O'Mahony, 2017; Kasinos et al., 2014; McSweeney, Healy, & Mulvihill, 2008). Similarly, the use of lecithin in conjunction with plant proteins has been reported to reduce the droplet size of oil-in-water emulsions. Emulsions containing 0.5% oil, 1% pea protein and 1% lecithin had mean particle size of 153 nm, which was lower than that measured for emulsions containing only lecithin (202 nm) or pea protein (234 nm) (Walia & Chen, 2020).

5. Conclusions

This review has provided a comprehensive and integrated analysis of inter-relationships between the chemical composition, physicochemical properties, functional properties and applications of lecithin across micelles, liposomes and oil-in-water emulsion systems. Lecithin ingredients from different botanical sources, including soybean, sunflower, rapeseed, corn and rice bran are co-products from vegetable oil processing, with complex chemical composition and important techno-functional properties. The functional properties of lecithin in micelles, liposomes and oil-in-water emulsions are strongly influenced by the physicochemical properties of PL. For instance, in lecithin, PC plays a fundamental role in the functional properties of a wide range of food structures and it has been shown that lecithin with a high proportion of PC facilitates the formation of emulsions with smaller particle size. However, the presence of other PL modifies the functional properties of lecithin, and therefore, only by knowing the relevant, detailed chemical composition of lecithin, it is possible to tailor its use in selected applications. The second most abundant component of lecithin is the fatty acids and it has been shown that saturated fatty acids are able to form highly stable liposomes. Together with fatty acids and PL, minor compounds present in lecithin, such as minerals and proteins, may modify the physicochemical properties of colloidal systems; however, only limited information is currently available from this area of research.

Concerning the ability of lecithin and, in particular PL, to form food structures, current research mainly focuses on oil-in-water emulsions and less information is available on micelles and liposomes. More data on the technological and formulation strategies to obtain food structures such as micelles and liposomes are necessary, and this would be of particular value for the incorporation of such structures in food and pharmaceutical applications. In addition, the strong technological functionality of lecithin presents opportunities for exploration in new food structures such as oleogels, which are emerging as fat mimetics. This literature review, by reporting the most relevant information in the area of functional properties of lecithin from botanical sources, and linking them with the physicochemical properties of the PL constituents, will help formulation scientists to best select lecithin across a wide range of food formulations and to scientifically underpin the interactions between lecithin and other ingredients present in selected applications.

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